# THE ULTRASTRUCTURE OF SPORES OF *COOKSONIA PERTONI*

*by* D. EDWARDS, K. L. DAVIES, J. B. RICHARDSON *and* L. AXE

ABSTRACT. The ultrastructure of spores isolated from sporangia of *Cooksonia pertoni* from Přídolí and Lochkovian rocks of the Welsh Borderland has been elucidated using both scanning and transmission electron microscopy. The Silurian material, attributable to *C. pertoni* subsp. *synorispora*, contains *Synorisporites verrucatus* while the Devonian *C. pertoni* subsp. *apiculispora* yields either *Streelispora newportensis* or *Aneurospora* sp. All three spore taxa show an exospore (≡ exine) composed of two layers, both of which extend into the murornate and verrucate sculpture in *Synorisporites verrucatus*, into the papillae and proximal folds of *Streelispora newportensis*, and into apertural folds (where observed) in all three taxa. In contrast, only the outer layer occurs in the apiculate sculpture of the Devonian spores. The homology of the layers is briefly discussed in relation to extant vascular cryptogams. Such ultrastructural observations compare favourably with published descriptions of the same taxa recorded from dispersed assemblages using light microscopy. Similar ultrastructure has already been recorded in cf. *Ambitisporites* isolated from Přídolí *Cooksonia pertoni* from Long Mountain, Shropshire, thus providing support for the hypothesis that, while the gross morphology and spore structure of *C. pertoni* remained unchanged from the Silurian into the Devonian, spore sculpture evolved from smooth to verrucate to apiculate.

THE record of early land plants in Silurian and basal Devonian rocks is largely based on small fragmentary coalified fossils of simple morphology and lacking anatomical detail. Studies on the use of *in situ* spores in attempts to deduce relationships and to detect hidden diversity are in their infancy. Thus the morphologically simple taxon *Cooksonia pertoni* Lang has been shown to possess at least three different kinds of spore depending in part on its geological age (Fanning *et al.* 1988). These are the dispersed miospore genera *Ambitisporites*, *Synorisporites* and *Streelispora/Aneurospora*, taxa that are united in their equatorially crassitate structure, but that differ mainly in the nature of their distal exines with sculpture being laevigate, verrucate or apiculate. From the relative ages of the megafossils and data from dispersed spore assemblages, it was inferred that laevigate spores (*Ambitisporites*) represent the ancestral state in *C. pertoni*, that apiculate spores are the most derived, and that the murornate-verrucate sculpture of *Synorisporites verrucatus* possibly represents an intermediate state in the *Ambitisporites-Streelispora/Aneurospora* lineage. *Streelispora/Aneurospora* is so written because limited numbers prevented a study and description of the proximal features of the *in situ* spores in all cases, and it is the presence or absence of proximal folds, that is used to distinguish the two genera in the dispersed record. In more recent work (Edwards *et al.* 1992) we have unequivocal evidence for *in situ Streelispora newportensis* in Lochkovian *C. pertoni*, and, in this study, for papillate apiculate equatorially crassitate spores lacking proximal folds, which can be more confidently assigned to *Aneurospora* than in previous studies, although there remain reservations on identification at specific level. Previous descriptions of the *in situ* spores employed light and scanning electron microscopy. Here we report on ultrastructure as seen in sections viewed by transmission electron microscopy.

## LOCALITY DATA, MATERIAL AND TECHNIQUES

All material is housed at the National Museum of Wales, Cardiff (NMW).

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*Ludford Lane, Shropshire* [*SO 5122 7412*]. Platyschisma Shale Member, Downton Castle Formation; *tripapillatus-spicula* Sporomorph Assemblage Zone; Přídolí Series, Silurian.

Two coalified discoidal sporangia (NMW 93.143G.1 and 2; Pl. 1, figs 1–5) containing *Synorisporites verrucatus* were recovered after bulk maceration, in 40 per cent. hydrofluoric acid, of a fossiliferous siltstone *c.* 1·6 m above the main bone bed at the famous locality at Ludford Lane Corner. This was the horizon that yielded the earliest terrestrial arthropods and stoma (Jeram *et al.* 1990) and is of early Přídolí age. The discoidal structures resemble *C. pertoni* in shape, with each bearing a centrally placed ridge marking the attachment of the subtended axis. Except at this site and at the periphery, where spores are occasionally visible, the surface of each specimen is covered by an acellular sheet with irregular depressions, interpreted as the remains of the sporangial cuticle. We therefore have neither direct gross morphological nor anatomical evidence for affinity with *C. pertoni*, identification being based on overall shape of the spore-containing region and the fact that *Synorisporites verrucatus* has not to date been found in a sporangium of any other shape.

*Brown Clee Hill.* Beneath small waterfall in stream section to the north of Brown Clee Hill, Shropshire; Ditton Group; lower middle part of *micrornatus-newportensis* Sporomorph Assemblage Zone; Lochkovian Stage, Lower Devonian.

The sporangia were isolated by disaggregation of the grey siltstone in water and cleaned by brief immersion in 40 per cent. hydrofluoric acid. They come from the upper plant-bearing horizon in the stream (Edwards *et al.* 1994). Dispersed palynomorphs indicate an early Lochkovian age (lower middle *micrornatus-newportensis* Zone). Four samples containing *Streelispora newportensis* were sectioned. Two are unequivocal sporangia. The less compressed one (NMW 93.143G.6) was initially almost intact distally, a fracture near the margin revealing cellular details of the sporangial wall and spores. In the second (NMW 93.143G.5) a short length of the subtending axis remains, but more of the distal wall has disappeared exposing the spores (Pl. 1, fig. 7). The remaining two are more compressed with preservation reminiscent of that in the Silurian examples: NMW 93.143G.3 has an almost circular outline; NMW 93.143G.4 is more fragmentary with an irregular outline (Pl. 1, fig. 6).

*M50 motorway* [*SO 6650 2658*]. Near 29·5 marker post on north side of motorway, Hereford and Worcester; St Maughans Formation; lowest part of *micrornatus-newportensis* Sporomorph Assemblage Zone; Lochkovian Stage, Lower Devonian (details in Edwards and Rose 1984).

The sectioned example (NMW 93.143G.7) was a spore mass, more or less circular in outline (Pl. 2, fig. 3), recovered from bulk maceration of a grey-green shale 2 m above the base of the St Maughans Formation. The distally apiculate, proximally papillate spores (Pl. 2, figs 4–7), lacking any proximal folds, are assigned to *Aneurospora* sp. and are believed to be derived from a *C. pertoni* sporangium since no other Lower Devonian sporangium of this shape has yet been demonstrated to contain *Aneurospora*.

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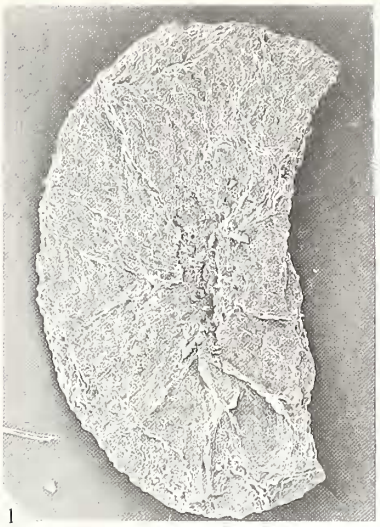
EXPLANATION OF PLATE 1

Figs 1–5. Scanning electron micrographs (SEMs) of *Cooksonia pertoni* subsp. *synorispora*; NMW 93.143G.1; Ludford Lane, Shropshire; Downton Castle Sandstone Formation, Přídolí Series (Upper Silurian). 1, lower side of sporangium showing spores at margins and site of axis attachment; × 44. 2, close up of outer surface of sporangium wall; × 210. 3, spores (*Synorisporites verrucatus*) before acid treatment, with remnants of wall on left; × 1000. 4, proximal surface of spore after acid treatment; × 3450. 5, part of distal surface of spore after nitric acid treatment; × 5570.

Fig 6–8. SEMs of *C. pertoni* subsp. *apiculispora*; north Brown Clee Hill, Shropshire; Ditton Group, Lochkovian (Lower Devonian). 6, NMW 93.143G.4; incomplete sporangium with visible spores and short subtending axis disintegrating on lower surface; × 25. 7, NMW 93.143G.5; sporangium with traces of axis; × 55. 8, NMW 93.143G.3; apiculate sculpture on distal surface of *Streelispora newportensis* after nitric treatment; × 3200.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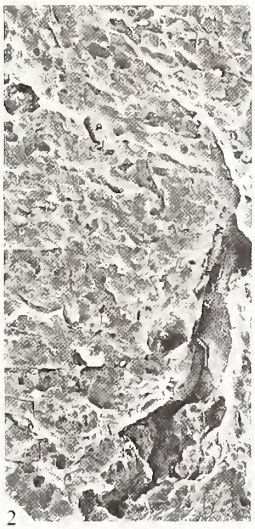
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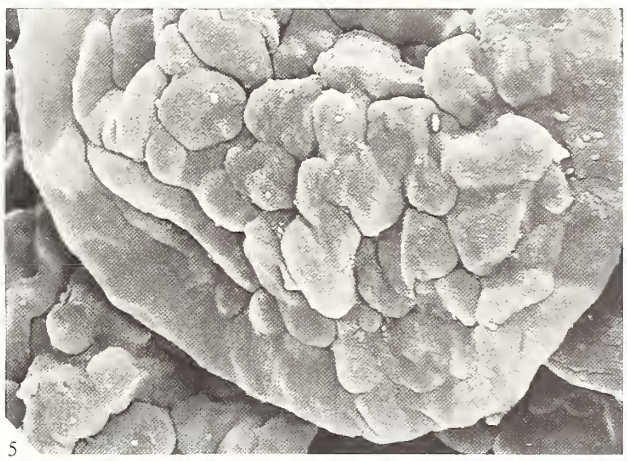
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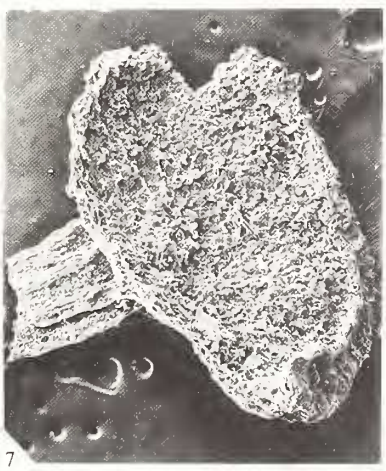
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All specimens were first examined untreated by SEM (Cambridge 360), to allow secure identification. In the early stages of the study, suitable material was then treated with fuming nitric acid to facilitate observations by LM but more importantly to remove pyrite prior to sectioning for TEM. This contrasts with methodology for dispersed spores, where only minimal nitric acid treatment is used to aid clearing. Indeed, our experiences with *Ambitisporites*, from a Přídolí *Cooksonia pertoni* from Long Mountain, Shropshire (Rogerson *et al.* 1993), indicated that valuable information was being destroyed by the concentrated acid treatment and our procedures were subsequently modified to include sectioning of untreated spores and examination by SEM after acid treatment. This was impossible on one of our original specimens (NMW 93.143G.5) where all material had already been used. In this study, therefore, sections were prepared from untreated spores where available and all material was subjected to fuming nitric acid for 30 minutes. Unlike the *Ambitisporites* spore masses from Long Mountain, those treated with nitric acid retained their integrity. Detailed techniques for sectioning for TEM are given in Rogerson *et al.* (1993). Essentially acid-treated and untreated specimens were dehydrated, embedded in Spurr resin, sectioned at 60–90 nm using an LKB Ultrotome 8801A and stained with 1 per cent. (w/v) potassium permanganate in 0·1 M phosphate buffer pH 6 followed by 2 per cent. (w/v) uranyl acetate and basic lead citrate. All sections were examined using a JEOL 100S TEM at an accelerating voltage of 80 kV.

## TERMINOLOGY

To avoid confusion, particularly with conventional palynological descriptions, the terminology adopted in our ultrastructural studies (Rogerson *et al.* 1993) is repeated. Observations on extant pteridophytes reveal three components of the sporoderm (see e.g. Tryon and Lugardon 1991). These are: *perispore*, the acetolysis-sensitive peripheral envelope; *exospore*, the acetolysis-resistant component largely composed of sporopollenin (exine *sensu* Potonié and Kremp 1954); and the *endospore*, laid down immediately outside the cell membrane and, being composed predominantly of cellulose, considered unlikely to survive taphonomic processes. Similarly the perispore (or perisporium; see Traverse 1988) *sensu stricto* rarely persists in fossils, but the term is sometimes used by palynologists for a loosely attached outer layer showing no infrastructure. In this study, we use the term 'peripheral layer' for extra-exospore coalified material. The trilete mark is usually represented in TEM by a projecting apertural fold, mainly involving the exospore. A superficial suture is rarely visible in SEM. Variations within the exospore such as differences in texture, staining or structure are termed layers.

## DESCRIPTIONS OF *IN SITU* SPORES

### Synorisporites verrucatus *Richardson and Lister, 1969*

Dimensions, means and sample numbers from the two sporangia are presented in Text-figure 1. A spore mass with abundant *S. verrucatus* recovered on bulk maceration from the type locality of *C. pertoni* at Perton Lane is interpreted as a possible coprolite because of its atypical shape and the

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EXPLANATION OF PLATE 2

Figs 1–2. Scanning electron micrographs (SEMs) of *Streelispora newportensis* before acid treatment showing papillae and folds; north Brown Clee Hill, Shropshire; Ditton Group, Lochkovian (Lower Devonian). 1, NMW 93.143G.4; × 3000. 2, NMW 93.143G.3; × 2080.

Figs 3–7. SEMS of spore mass of *C. pertoni* subsp. *apiculispora* shape, containing *Aneurospora* sp. before acid treatment; NMW 93.143G.7; M50 motorway, Hereford and Worcester; St Maughans Group, Lochkovian (Lower Devonian). 3, spore mass; × 95. 4, group of spores; × 1575. 5, sculpture on distal surface; × 2650. 6, close up of proximal face with pitted appearance; × 3100. 7, proximal face with pronounced apertural folds and interradial papillae; × 2900.


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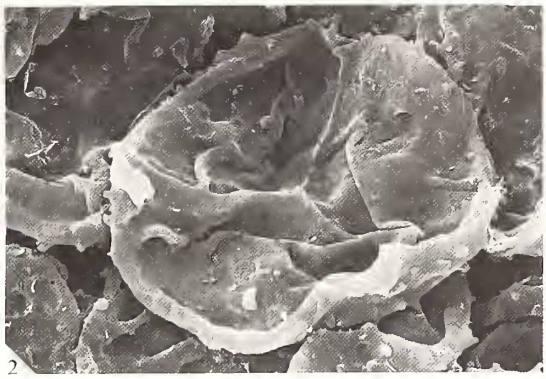
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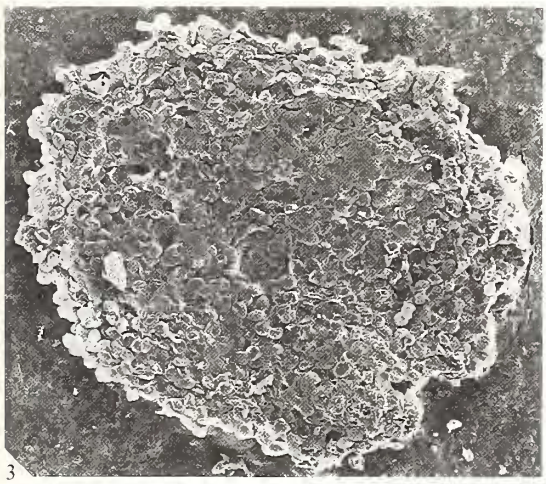
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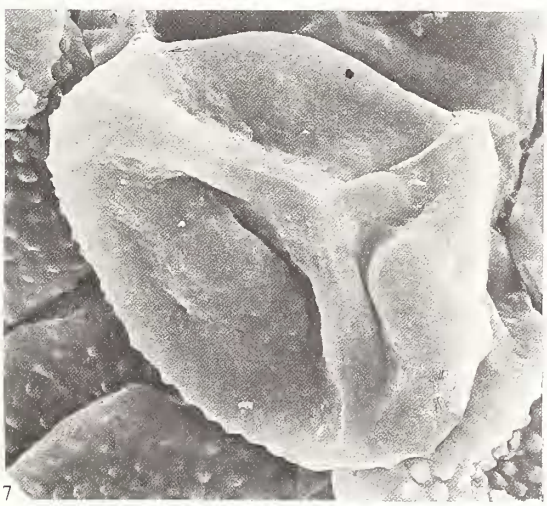
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EDWARDS *et al.*, *Streelispora*, *Cooksonia*

| CHARACTER | | | TAXON | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Synorisporites verrucatus* | | | | | |
| | | | NMW 93.143G.1 ex Ludford Lane | | | NMW 93.143G.2 ex Ludford Lane | | |
| Dimensions of sporangium (mm) | | | 1.48 x 1.01 | | | 0.89 x 0.95 | | |
| | | | min. | mean | max. | min. | mean | max. |
| Diameter of spore minus acid (μm) | | | 14.00 | 19.76 (n=23) | 22.06 | 16.80 | 23.04 (n=5) | 27.20 |
| Diameter of spore plus acid (μm) | | | 16.66 | 20.70 (n=28) | 27.27 | 16.60 | 24.28 (n=12) | 33.30 |
| Width of peripheral layer (μm) | | | 0.025 (n=60) | 0.12 [minus acid] | 0.33 | 0.025 (n=27) | 0.10 [minus acid] | 0.20 |
| Width of outer exosporal layer (μm) | | | 0.10 | 0.27 (n=112) | 1.10 | 0.04 | 0.05 (n=66) | 0.08 |
| Width of inner exosporal layer (μm) | | | 0.33 | 1.15 (n=89) | 2.60 | 0.44 | 0.96 (n=49) | 1.93 |
| Width of dark layer around lumen (μm) | | | 0.030 | 0.033 (n=47) | 0.060 | 0.020 | 0.040 (n=40) | 0.130 |
| Dimensions of distal sculpture (μm) | TEM data | Height | 0.50 | 1.11 (n=17) | 2.00 | 0.20 | 0.52 (n=5) | 1.00 |
| | | Width at base | 1.00 | 2.81 (n=17) | 3.66 | 1.28 | 1.86 (n=5) | 2.62 |
| | SEM data | Height | 0.68 | 1.08 (n=10) | 1.59 | 1.57 | 1.99 (n=10) | 3.15 |
| | | Width at base | 0.90 | 1.36 (n=10) | 1.59 | 2.10 | 2.50 (n=13) | 3.15 |

TEXT-FIG. 1. Quantitative data for *Synorisporites verrucatus*.

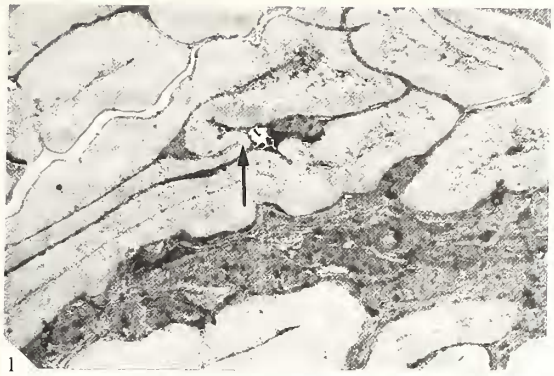
presence of at least two further spore taxa. However, the ultrastructure of unequivocal *S. verrucatus* from the mass is consistent with that in the Ludford Lane samples.

Under the scanning electron microscope, the spores look similar both before and after acid treatment (Pl. 1, figs 3, 5). Untreated examples were usually visible at the edges of the mass, some still as components of tetrads (Pl. 1, fig. 3). Distal surfaces show typical verrucate to murornate

EXPLANATION OF PLATE 3

Figs 1–6. Transmission electron micrographs (TEMs) of sections of *Synorisporites verrucatus*; 1–3, 5–6, NMW 93.143G.1; 4, NMW 93.143G.2; Ludford Lane, Shropshire; Downton Castle Sandstone Formation, Přídolí Series (Upper Silurian). 1, sections through a number of spores, before nitric acid treatment, with extensive darker intersporal material; arrow indicates possible apertural fold; × 5840. 2, as for 1 but with possible aborted spores which are narrower and darker; arrow indicates pronounced black peripheral layer; × 9890. 3, as for 1 and 2 but with well developed black peripheral layer; narrow 'fuzzy' line marks position of lumen; × 16690. 4, narrow innermost layer of exospore following nitric acid treatment adjacent to lumen (light central area); × 27360. 5, remnant of membrane-like material or remains of aborted spores following treatment with nitric acid; × 350. 6, section through both surfaces of single spore after acid treatment, but with lumen barely visible as discontinuous white line; note prominent outer layer of exospore; × 9170.

Figs 7–10. TEMs of sections of *Streelispora newportensis* spores; NMW 93.143G.4; north Brown Clee Hill, Shropshire; Ditton Group, Lochkovian (Lower Devonian); see Pl. 1, fig. 6, Pl. 2, fig. 1. 7, number of spores before acid treatment; arrow indicates possible apertural fold; × 9030. 8, proximal and distal surface of same spore after acid treatment showing pronounced outer layer of exospore which is thicker and extends into sculpture on distal surface; part of a second spore is seen top right; × 14370. 9, possible detached peripheral layer; × 21190. 10, as for 8 but with dark innermost layer of exospore marking the lumen; × 30450.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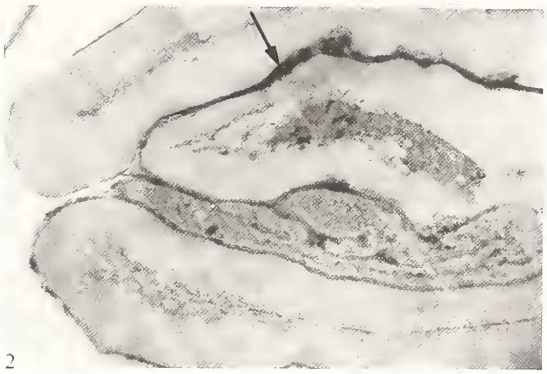
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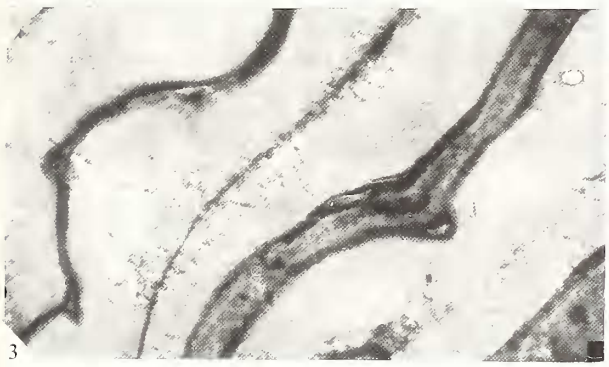
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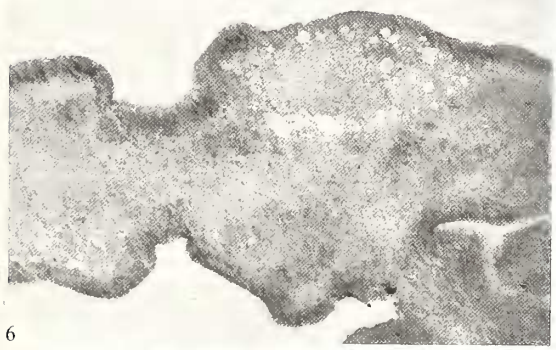
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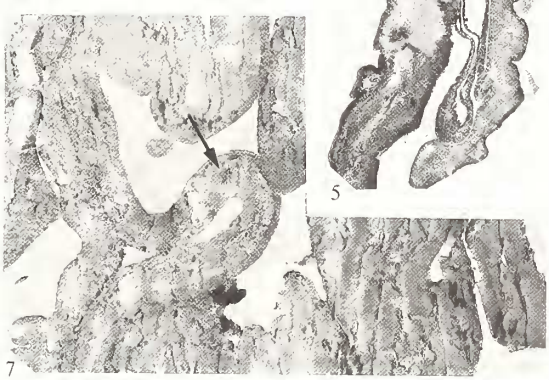
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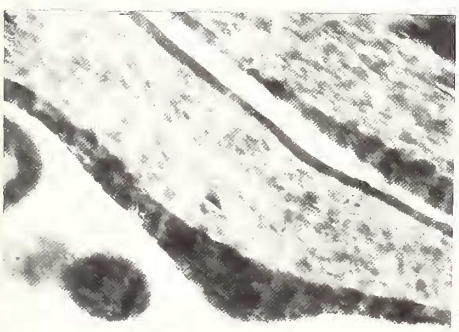
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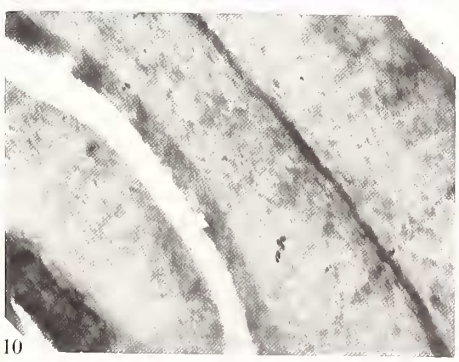
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EDWARDS *et al.*, *Synorisporites*, *Streelispora*

sculpture (Pl. 1, fig. 5); the proximal surface is smooth (Pl. 1, fig. 4) as is the equatorial crassitate region. The trilete mark is prominent, each arm being represented by a ridge lacking sutures and extending to the equator. Interradial areas may be folded and always lack papillae (Pl. 1, fig. 4).

In contrast, TEMs look markedly different before and after nitric acid treatment. Untreated spores are tightly adhered in the spore mass (Pl. 3, figs 1–3), but are encompassed by an intersporal matrix, readily distinguished from the very narrow darker region (≡ peripheral layer of Rogerson *et al.* 1993) immediately surrounding individual spores (Pl. 3, figs 2–3). This intersporal region is more or less homogeneous apart from narrow 'membranous' lines, sometimes describing irregular flattened oval outlines, sometimes disjunct (Pl. 3, fig. 2). These structures usually survive treatment with nitric acid, but the remainder of the intersporal matrix does not (Pl. 3, fig. 5). The peripheral layer, present to varying degrees and of irregular thickness as it 'follows' the contours of the exospore, also disappears on acid treatment. In light microscopy it appears as a coalified layer, obscuring further detail. The wall surviving fuming nitric acid treatment is interpreted as the exospore and, in this state, comprises two or possibly three layers (Pl. 3, figs 4, 6). An outermost dark electron-dense layer of more or less uniform thickness (termed outer exospore) surrounds a wider lighter homogeneous layer (under exospore), both of which are visible in the sculpture (Pl. 3, fig. 6). Groups of circular to oval electron transparent cavities occur largely throughout the homogeneous layer but may extend into the outer exosporal layer (Pl. 3, fig. 6). The innermost narrow dark layer immediately around the lumen is more variable in thickness and sometimes absent (Pl. 3, figs 4, 6). It is more clearly defined and prominent in specimen NMW 93.143G.2 (Pl. 3, fig. 4). In untreated specimens the lumen is represented by a dark line. Acid treatment induces separation of the spore walls so that the lumen becomes visible. The lumen also extends into the apertural fold (Pl. 3, fig. 1), in which all layers of the exospore are visible.

Streelispora newportensis (*Chaloner and Streel*) *Richardson and Lister, 1969*

Quantitative data from the four samples, all from north Brown Clee Hill, are presented in Text-figure 2. Scanning electron micrographs before and after acid treatment look similar. Intersporal variation relates mainly to proximal features; almost all show papillae, but associated folds may be variously orientated, i.e. not consistently periclinal, with additional folding sometimes present (Pl. 2, figs 1–2). Distal ornament of coni varies only in size (Pl. 1, fig. 8). In all specimens prior to nitric acid treatment, the outlines of individual spores are difficult to observe under TEM since spores are tightly adpressed and consequently difficult to section. Therefore, the existence of a peripheral layer is equivocal. However, less compressed untreated spores were occasionally observed under TEM. Such spores exhibit distinct layering of the exospore (Pl. 3, fig. 7). After exposure to acid the spores separate and the profiles become well-defined. Traces of a thin dark layer (?peripheral layer) remain in one specimen, although this may also be an atypical example of sloughing of the outer exospore (Pl. 3, fig. 9), and there is scant evidence for an intersporal mix. The exospore has two conspicuous layers with a narrow more electron-dense layer surrounding a wider homogeneous one (Pl. 3, fig. 10). Both layers are present in the proximal folds (Pl. 4, figs 1–3), apertural folds and papillae (Pl. 4, fig. 2), but only the outer is seen in the ornament (Pl. 3, fig. 8; Pl. 4, figs 1, 5). A very dark line delimits the lumen (Pl. 3, fig. 10), and may be associated with ovoid electron-dense bodies (0·66–2·00 $\mu$m × 0·50–0·66 $\mu$m diameter). As in *Synorisporites verrucatus*, this line is not visible in all sections and varies in thickness.

Aneurospora *sp.*

The sporangium of *C. pertoni* shape (Pl. 2, fig. 3) contained proximally tripapillate, equatorially crassitate, apiculate spores (Pl. 2, fig. 7), assigned to *Aneurospora* in that proximal folding is lacking. Dimensions are given in Text-figure 2. A thin, darkly stained peripheral layer is apparent under TEM (Pl. 4, fig. 9) and only partially disappears on exposure to acid (Pl. 4, fig. 6). The exospore itself is two-layered (Pl. 4, figs 6, 10–11) with the outer narrow electron-dense layer contrasting with the wide homogeneous inner, the latter being relatively wider than that in *Streelispora*. Both layers

| CHARACTER | | | TAXON | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Streelispora newportensis* | | | *Aneurospora* | | |
| | | | Pooled data for NMW 93 143G 3-6 ex Brown Clee Hill | | | NMW 93.143G.7 ex locality DE 98 M50 | | |
| Dimensions of sporangium/spore mass (mm) | | | 1.43 x 1.0 (30.1 series) 1.95 x 1.87 (40.1 series) 1.14 x 0.68 (UF1566 series) 0.93 x 0.79 (55.6 series) | | | 0.78 x 0.67 | | |
| | | | min. | mean | max. | min. | mean | max. |
| Diameter of spore minus acid (µm) | | | 13.64 | 21.56 (n=32) | 28.38 | 18.97 | 24.52 (n=22) | 28.00 |
| Diameter of spore plus acid (µm) | | | 15.00 | 22.81 (n=26) | 31.20 | | - | |
| Width of peripheral layer (µm) | | | 0.12 | 0.18 (n=5) | 0.36 | 0.03 | 0.04 (n=34) | 0.11 |
| Width of outer exosporal layer (µm) | | | 0.06 | 0.25 (n=273) | 0.55 | 0.03 | 0.06 (n=42) | 0.12 |
| Width of inner exosporal layer (µm) | | | 0.16 | 0.65 (n=205) | 1.87 | 0.40 | 0.99 (n=42) | 1.68 |
| Width of dark layer around lumen (µm) | | | 0.02 | 0.04 (n=48) | 0.12 | 0.05 | 0.11 (n=23) | 0.35 |
| Dimensions of proximal fold (µm) | | Height | 0.86 | 1.44 (n=9) | 3.66 | | - | |
| | | Width at base | 0.46 | 0.73 (n=9) | 1.33 | | - | |
| | | Width at apex | 0.33 | 0.52 (n=5) | 0.80 | | - | |
| Dimensions of proximal papilla (µm) | | Height | 1.39 | 1.98 (n=8) | 2.66 | 1.29 | 2.17 (n=7) | 2.22 |
| | | Width at base | 1.25 | 3.85 (n=8) | 6.00 | 3.22 | 4.70 (n=7) | 5.80 |
| | | Width at apex | 0.62 | 2.87 (n=3) | 4.00 | | - | |
| Dimensions of distal sculpture (µm) | TEM data | Height | 0.33 | 0.77 (n=21) | 1.20 | 0.25 | 0.43 (n=10) | 0.95 |
| | | Width at base | 0.73 | 1.44 (n=20) | 2.13 | 0.25 | 0.72 (n=10) | 0.93 |
| | SEM data | Height | 0.45 | 0.82 (n=20) | 0.93 | 0.51 | 0.63 (n=10) | 1.02 |
| | | Width at base | 0.69 | 1.01 (n=20) | 1.39 | 0.51 | 0.83 (n=15) | 1.02 |

TEXT-FIG. 2. Quantitative data for *Streelispora newportensis* and *Aneurospora*.

are present in the apertural fold and papillae (when present) with only the outermost layer in the distal coni (Pl. 4, fig. 11). TEMs reveal that the projecting coni frequently coincide with depressions in the exospore of adjacent spores. It is a possibility that the pitted appearance of the latter when observed under SEM (Pl. 2, fig. 6) is due to the impaction of coni on neighbouring spores during compression, or may merely be due to localized corrosion.

## DISCUSSION

### *Effects of nitric acid treatment*

*Layering within the exospore.* The striking differences noted in cf. *Ambitisporites* spores from *Cooksonia pertoni* sporangia before and after acid treatment allowed interpretation of observed ultrastructure in terms of the original chemistry of the peripheral layer and the exospore. However, it raised the possibility that the layering of the exospore, particularly prominent after staining, is actually produced by acid treatment (Rogerson *et al.* 1993). The same kind of layering, present in all the acid-treated spores in this study, demands similar assessment as a prerequisite for comparisons of exospore structure in extant and extinct embryophytes. Is the nitric acid accentuating original differences in the wall and their affinities for the stain, or is the layering merely reflecting the degree of penetration of the nitric acid (and hence chemical modification) into the spore wall? Evidence for the latter comes from the consistently peripheral position of the staining and its more or less uniform width. It could be argued that the minor variations relate to timing of the various procedures and/or degree of penetration of the resin. On the other hand, again based on a relatively small sample size, for *Streelispora* and *Synorisporites*, the thickness of the outer layer relative to that of the exospore is more or less constant. Further, in considering the apiculate ornament of *Aneurospora* and *Streelispora*, the area of staining is greater than that anticipated from the degree of penetration into the rest of the wall. Perhaps most importantly, the layering is not a feature of all spores treated with nitric acid (see e.g. *Synorisporites downtonensis* illustrated in Text-fig. 3A–B) and is frequently observed in non-treated spores. Further layering has been recorded and illustrated in specimens of *Ambitisporites*, *Synorisporites*, *Streelispora* and *Aneurospora* studied using light microscopy (Richardson and Ioannides 1973, p. 14).

The differing intensity of staining observed between samples and occasional 'reversed staining' effects may well reflect minor differences in procedures, but even where there is little or no contrast between layers, the outer consistently appears smoother and the middle more granular.

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EXPLANATION OF PLATE 4

Figs 1–5. Transmission electron micrographs (TEMs) of sections of *Streelispora newportensis* after fuming nitric acid treatment; 1–3, NMW 93.143G.4; 4–5, NMW 93.143G.6; north Brown Clee Hill, Shropshire; Ditton Group, Lochkovian (Lower Devonian). 1, number of spores stacked in sporangium; lumens are not visible, arrow indicates possible proximal fold; × 5940. 2, part of spore with papilla and fold in section on proximal surface and sculpture on distal; lumen is not visible; × 10490. 3, fold on proximal surface and some extrasporal material; × 20000. 4, possible equatorial thickening; note differing response of layers of the exospore to sectioning and 'reversed' staining of middle and outer layers; × 9200. 5, sculpture comprising outer layer of exospore; × 12400.

Figs 6–11. TEMS of sections of *Aneurospora* sp. following concentrated nitric acid treatment, all except fig. 9 which is before acid treatment; note outer layer of exospore is here stained lighter than remainder of wall; see NMW 93.143G.7 (Pl. 2); M50 motorway, Hereford and Worcester; St Maughans Group, Lochkovian (Lower Devonian). 6, outer and inner layers, lumen and possible peripheral layer; × 20570. 7, apertural fold, triangular area with extended 'arms' marks lumen; × 4360. 8, poorly developed equatorial thickening; × 5960. 9, distal surface showing sculpture with peripheral layer before acid treatment; × 22000. 10, section through distal and proximal surface with some evidence of innermost exospore layer visible as a dark line around oblique lumen; × 22225. 11, distal surface with sculpture formed from outer layer only; some evidence for a particulate peripheral layer; × 27780.


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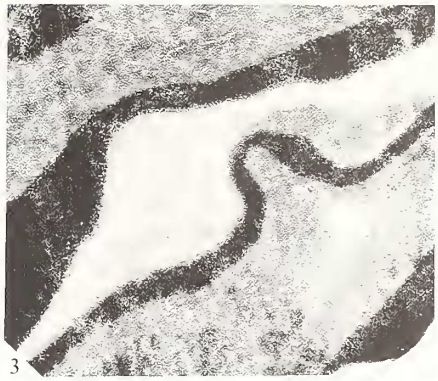
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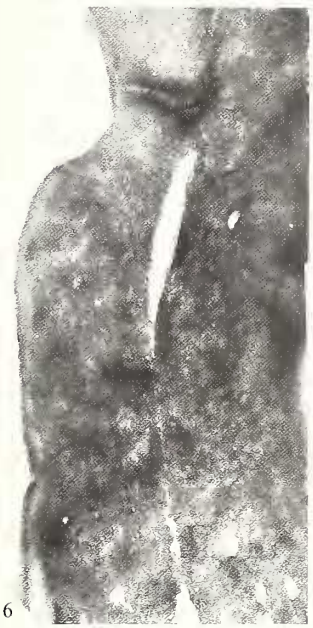
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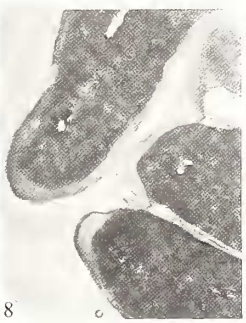
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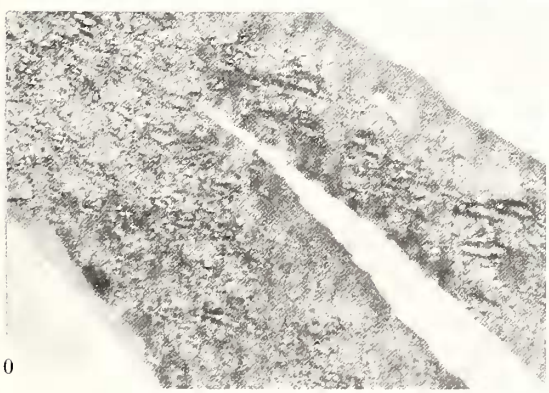
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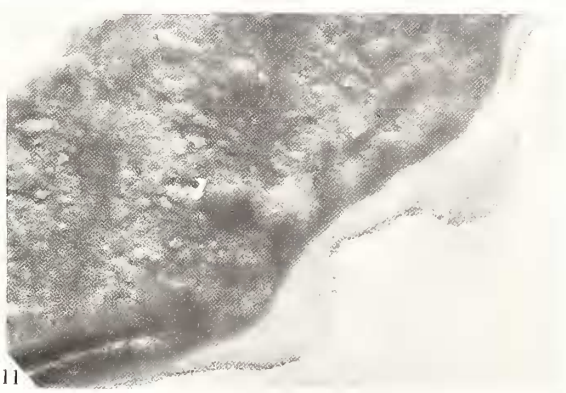
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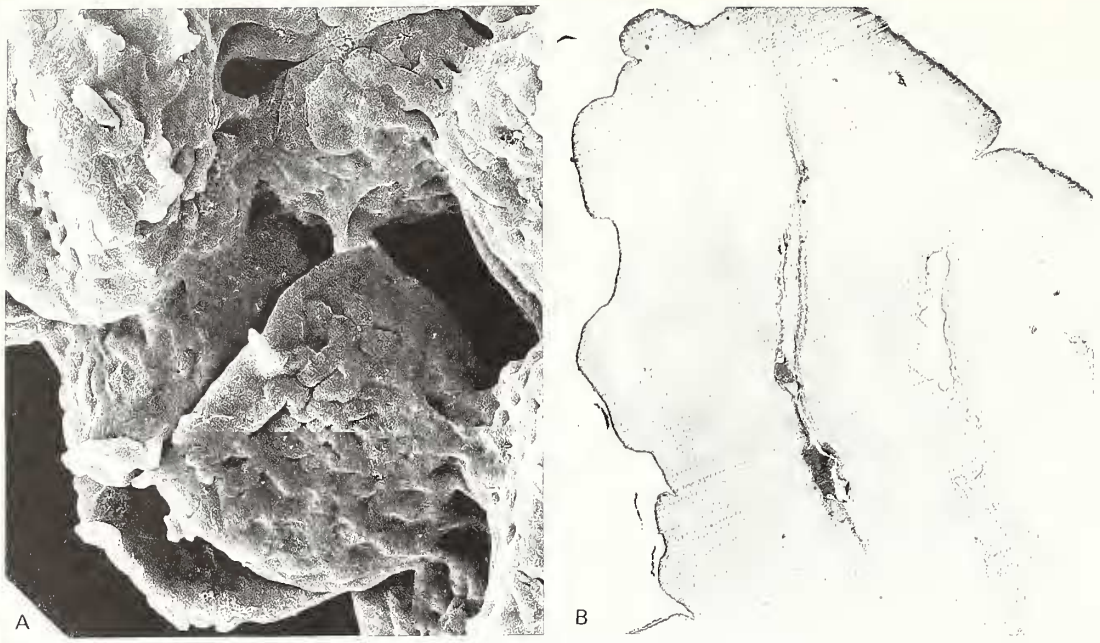
EDWARDS *et al.*, *Streelispora*, *Aneurospora*




TEXT-FIG. 3. A, SEM showing tetrads of *Synorisporites downtonensis* (NMW 93.143G.8), Ludford Lane, Přídolí Series, × 792. B, TEM of section of thick-walled spores after nitric acid treatment (NMW 93.143G.8), × 3352.

Assuming then that the nitric acid is revealing information on wall layering, what is the basis for this response? Possibilities include chemical differences relating to polymerization of sporopollenin or physical differences involving variation in the original substructures on which sporopollenin is deposited. The differing responses of the exosporal layers of *Aneurospora* and *Streelispora* to the knife may well reflect differences in their physical properties. Thus, the inner layer appears chattered while the outer is unaffected (cf. Pl. 3, fig. 7; Pl. 4, figs 4–5). Whatever the cause, it is likely that differential compaction and homogenization during diagenesis would have obliterated any original fine structure, making comparisons with extant spores of limited value. Nevertheless, there are similarities with certain filicalean ferns, such as zonation with comparable dimensions (Lugardon 1990), and indeed Professor Lugardon, having seen our *Ambitisporites* material, was of the same opinion and wrote 'elles (i.e. the layers) correspondent probablement à des résistance à l'acide en relation avec des variations chimique, ou physico-chimique de la sporopollenine à l'intérieur d'une même couche'. He believes that the layers in living ferns correspond to the variation in degrees of polymerization of sporopollenin as layers are deposited during maturation, but which usually, but not consistently, have disappeared in mature spores. Similar atlases are needed for mosses and hepatics. In a brief overview (Brown and Lemmon 1990), two-layered exines are recorded in certain hepatics, hornworts and *Sphagnum* although the bryopsid exine is described as typically homogeneous. Such comparisons made on the 'nearest living match' approach are over simplistic and certainly are not intended to reflect relationship. The likelihood that spore wall ultrastructure has remained static over four hundred million years must be debated. However, surveys of extant filicaleans and a few bryophytes do show that exosporal layering is present and that its significance has been addressed in extant cryptogams.

*Peripheral layer*. Observations by light microscopy in *Ambitisporites* revealed the peripheral layer as an adhering coalified sheet, while its reaction with concentrated nitric acid indicated a different chemistry from the probably sporopollenin-impregnated exospore. Nitric acid had a similar effect

in the present investigations although traces of the enveloping dark layer remain in *Aneurospora* and may be equivalent to a translucent 'structureless' layer sometimes visible in light microscopy.

In contrast to the marked morphological differences noted by SEM in 'before and after' treatments of *in situ* cf. *Ambitisporites*, there is little change in the spores discussed here.

*Intersporal matrix*. This behaved in similar fashion to the peripheral layer, although traces of 'membranous' sheets remained after concentrated nitric acid treatment in *Synorisporites*.

*Size*. Although nitric acid is sometimes cited as increasing the size of spores, we have noted only a small increase in maximum diameter (< 5 per cent. in *Synorisporites*, 3 per cent. in *Streelispora*), but the small size sample and difficulties of measurement limit confidence in the data. There are no noticeable changes in wall thickness but, in a number of cases, lumens become apparent as distal and proximal walls separate. There is also the suggestion, particularly from SEMs that the exospore becomes more supple with collapse between unsupported areas, thus enhancing features such as crassitudes and apertural folds, although these are also visible on untreated material.

## HOMOLOGY OF SPORE LAYERS AND ASSOCIATED STRUCTURES

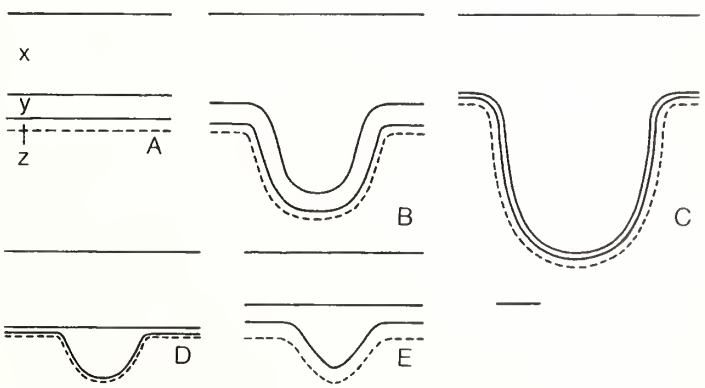
(Text-figure 4)

### *Exospore*

The concentrated nitric acid-resilient component is interpreted as exospore. Apart from differences in electron opacity, producing the layering, it is apparently homogeneous, although the possibility that its original structure has been eliminated during diagenesis cannot be discounted. The general appearance is similar to that recorded in cf. *Ambitisporites* (Rogerson *et al.* 1993). The very dark narrow line around the lumen seen in *Aneurospora* and *Synorisporites* is not a consistent feature, but, where present, persists after nitric acid treatment. It might represent the remains of spore contents or even endospore. Layering in an otherwise homogeneous exospore is seen in illustrations of extant ferns (Lugardon's 1990 Filicinées) and articulates and also in liverworts (Brown and Lemmon 1990) where associated with lamellar organization.

A suture on the trilete mark has never been observed in sections of *Ambitisporites*, *Synorisporites verrucatus*, *Streelispora newportensis* and *Aneurospora*. Its apparent presence in preparations viewed by light microscopy may be due to the lumen projecting into the apertural fold.

The electron-transparent circular to oval cavities noted in cf. *Ambitisporites* (Rogerson *et al.* 1993) are common in *Synorisporites verrucatus*, particularly after acid treatment where they may be localized towards the equator, less common in *Streelispora newportensis* and absent from *Aneurospora* sp. They occur in outer and inner layers of the exospore, and occasionally cross the junction between them. Similar structures are present in TEM preparations of *Parka* (Hemsley




TEXT-FIG. 4. Schematic representation of exospore layers in *Ambitisporites* sp. (A), *Synorisporites verrucatus* (B, C), *Aneurospora* sp. (D) and *Streelispora newportensis* (E). x = inner exospore, y = outer exospore, z = peripheral layer. Scale bar represents 0·52 μm.

1989), and are called 'bubble-like cavities'. It seems likely that they are artefacts of preparation or develop during preservation but we have no explanation for their formation.

*Peripheral layer and intersporal matrix*

The nature of the acid sensitive layer enveloping the exospore was somewhat inconclusively discussed above in relation to cf. *Ambitisporites* (Rogerson *et al.* 1993). It is comparable in position (Brown and Lemmon 1990) and reaction to concentrated nitric acid with the perispore recorded in mosses, ferns, Lycopodiaceae, and microspores of heterosporous lycophytes (Lugardon 1990) where it is deposited by condensation of tapetal particles onto one or several layers, after completion of the exospore. However, the perispore of extant plants is highly ordered, sometimes with taxon-specific surface patterning and seems to comprise a far more discrete layer than that recorded here. We therefore remain equivocal on the homology of the peripheral layer with the perispore. We are also uncertain of its relationship to the far more extensive intersporal 'matrix' recorded in *Synorisporites* which may be distinguished from the peripheral layers because of its more granular appearance and lighter staining. Like the peripheral layer it disappears on treatment with fuming nitric acid. The membrane-like structures which withstand acid treatment may well be the remains of aborted spores and have outlines consistent with this hypothesis. A further possibility is that they are similar to the ornamented sheets noted between spores of *Uskiella spargeus* (Shute and Edwards 1989) and compare with sheets formed by fusion of Ubisch bodies in spermatophytes. The intersporal matrix may represent the remains of a periplasmodial tapetum, but more probably represents the locular fluid which bathes developing spores. Although a liquid has low fossilization potential, it may have been preserved in this case because of its colloidal or viscous nature, itself possibly due to the presence of more recalcitrant molecules as precursors of sporopollenin.

## COMPARISONS WITH RELEVANT DISPERSED SPORE TAXA

It is of some interest to compare the ultrastructural detail presented here with structure recorded in the diagnoses of the dispersed spores which were originally based on light microscope observations. Thus, considering the emended diagnosis of the genus *Streelispora*, Richardson *et al.* (1982) described the exine as 'two-layered (possibly three-layered)' with 'layers closely adpressed over most of the surface' and contact areas 'characterized by tangential folds and small radial folds of the outer thin exo-exinal layer'. For *S. newportensis* the papillate thickenings are considered part of a second and thicker underlying exo-exinal layer. Our observations confirm the layering, our outer and inner exospore being equivalent to the outer thin exo-exinal and underlying thicker exo-exinal layers respectively, but suggest that the inner exo-exinal layer also contributes to the folds (Text-fig. 4). From the nature of the proximal radial and tangential folds, as interpreted by light microscopy, it had been expected that the two layers were loosely attached on the proximal surface. However, none of the sectioned spores illustrated here show separation of the two layers with the outer, forming a fold. The major structural feature, 'the more or less equatorial crassitude', is surprisingly difficult to identify in our TEM sections, although it is present and best developed in spores from specimen NMW 93.143G.6 (Pl. 4, fig. 4). Likewise it is not pronounced in TEMs of *Synorisporites* or *Aneurospora*. Indeed in their emended generic diagnosis for the latter, Richardson *et al.* (1982) described a subequatorial region which is 'especially rigid and probably thickened so as to appear like a dark band (equatorial crassitude); the inner limits of it are often ill-defined and its width is also ±variable even in the same specimen'. This description fits very well with our observations. In all four genera (*Ambitisporites*, *Synorisporites*, *Streelispora* and *Aneurospora*), viewed under the light microscope, the equatorial crassitude sometimes appears as more rigid than the adjacent distal area and retains its shape in various compressional states.

In contrast, although, in the original description of *Synorisporites verrucatus* (Richardson and Lister 1969), the exine is described as homogeneous with the equatorial crassitude 2–3 μm wide, in later light microscope descriptions of better preserved *Synorisporites* (*verrucatus* and *tripapillatus*) and *Ambitisporites*, Richardson and Ioannides (1973, p. 277, pl. 5, fig. 5) showed a closely adherent

diaphanous outer layer. This is now thought to be equivalent to the outer exospore seen in our TEM sections.

## EVOLUTION IN *COOKSONIA* SPORES

Based on SEM studies and stratigraphical occurrences it was originally suggested that spores of *Cooksonia pertoni* all have a similar structure (i.e. equatorially crassitate) but that sculpture changed in time from smooth to verrucate to apiculate (Fanning *et al.* 1988). These TEM observations support the hypothesis as regards structure, and show how the distribution of the outermost layer varies with change in ornament. There is also a decline in total wall thickness excluding sculpture, although, because of the nature of its ornament, *Synorisporites verrucatus* spores appear to have larger amounts of sporopollenin than *Ambitisporites*. There is no evidence that the ornament in *S. verrucatus*, although superficially sometimes similar to surface wrinkling, was formed by contraction: the muri and verrucae were formed by additional material with resultant increase in surface area even though the actual diameter of the *in situ* spores of *S. verrucatus* is less than that for *Ambitisporites* sp.

Although our *in situ Aneurospora* and *Streelispora* came from strata of differing age, their apiculate sculpture is similar and shows little intrasporangial variation. Minor differences in appearance between the two relate to quality of preservation. Considering the dispersed spore record, although the two genera form only a small numerical proportion of assemblages, there is a large number of integradational forms based mainly on the type and distribution of apiculate/granulate sculpture. The various types of sculptural forms and the proportions of the variants present change throughout the Lochkovian, but as yet we have insufficient numbers of megafossils with *in situ* spores to relate this to the evolution of subspecies of *Cooksonia pertoni*.

A similar trend in exine morphology (namely, laevigate–verrucate–apiculate) has been recorded in dispersed spore representatives of a second structurally different miospore morphotype, although as yet the parent plants remain unknown. The trend is further exhibited by cryptospores, probably indicating a convergence in response to common environmental pressures in at least two major groups of land plants (Richardson and Burgess 1988).

Finally, a peripheral layer has been demonstrated in all four taxa, and is most persistent in *Aneurospora*, where traces remain after nitric acid treatment, but is frequently absent in *Streelispora*. This leads to questions relating to the relative maturity of these *in situ* spores and the possibility that *Cooksonia* sporangia containing *Aneurospora* spores with their thinner peripheral layer (specimen NMW 93.143G.7, Pl. 2, figs 3–7) are immature. Against the latter is the common occurrence of *Aneurospora* in the dispersed record.

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D. EDWARDS

K. L. DAVIES

L. AXE

Department of Earth Sciences
University of Wales College of Cardiff
Cardiff CF1 3YE, UK

J. B. RICHARDSON

Department of Palaeontology
Natural History Museum
London SW7 5BD, UK

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